New Constituents from Stems of *Artabotrys uncinatus*

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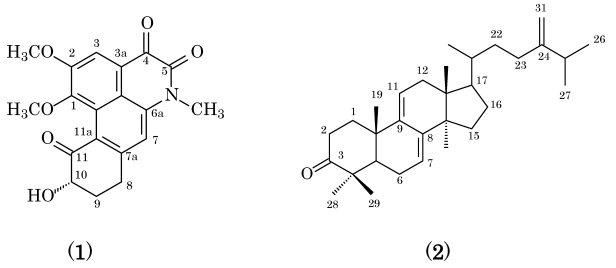
Two new compounds, 4,5-dioxoartacinatine (1) and 24-methylenelanosta-7,9(11)-diene-3-one (2), together with thirty known compounds were isolated and characterized from the stems of *Artabotrys uncinatus*. Structures of the new compounds were determined by spectral analysis.

Key words *Artabotrys uncinatus*; 4,5-dioxoartacinatine; biological assay; 24-methylenelanosta-7,9(11)-diene-3-one; antioxidant activity

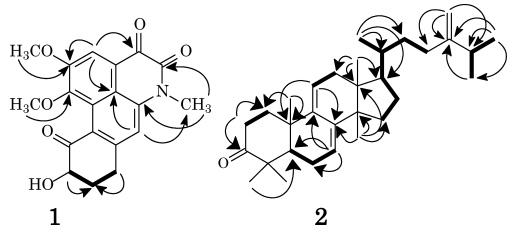
There are more than 100 species of the genus *Artabotrys* throughout tropical Africa and East Asia.[1)] *Artabotrys uncinatus* (LAM.) MERR. (Annonaceae) is widely distributed throughout southern Taiwan, and the roots and fruits are used for the treatment of malaria and scrofula.[2)] Previous literature has shown this genus to contain alkaloids, triterpenoids, lignans, flavonoids, and steroids.[2—6)] Among them, yingzhaosu analogues showed notable antimalarial activities *in vitro*[3)]; alkaloids showed cytotoxic and antithrombotic activitives.[5)] In this study, we investigated the stem parts of *A. uncinatus*, and two new compounds, 4,5-dioxoartacinatine (**1**) and 24-methylene lanosta-7,9(11)-diene-3-one (**2**), along with thirty known compounds: cloven-2β,9α-diol (**3**),[7)] caryolane-1,9β-diol (**4**),[7)] 1-methoxy-9-caryolanol (**5**),[8)] spathulenol (**6**),[9)] (−)-*ent*-4β-hydroxy-10α-methoxyaromadendrane (**7**),[10)] 4β-hydroxy-10α-methoxyaromadendrane (**8**),[10)] β-caryophyllene-8*R*,9*R*-oxide (**9**),[7)] artabotryside A (**10**),[11)] artabotryside B (**11**),[11)] apigenin (**12**),[12)] luteolin (**13**),[13] 5-hydroxy-7,4′-dimethoxyflavone (**14**),[14)] (+)-catechin (**15**),[15)] liriodenine (**16**),[2)] atherospermidine (**17**),[16)] artacinatine (**18**),[5)] (−)-asimilobine (**19**),[2)] (−)-artavenustine (**20**),[17)] *N*-*P*-coumaroyltyramine (**21**),[18)] (+)-syringaresinol (**22**),[19)] (2*R*,3*R*)-3-hydroxyl-2-methylbutyrolactone (**23**),[20)] tetrahydrofuran-4-methylidene-3-ol (**24**),[21)] phytol (**25**),[22)] 24-methylenelanosta-7,9(11)-dien-3β-ol (**26**),[23)] (24*R*)-stigmasta-5-en-3β,7α-diol (**27**),[24)] (22*E*,24*S*)-stigmasta-5,22-dien-3β,7α-diol (**28**),[24)] β-sitosterol (**29**) and stigmasterol (**30**), β-sitosteryl-3-*O*-β-D-glucoside (**31**), and stigmasteryl-3-*O*-β-D-glucoside (**32**) were isolated. Compounds **13**, **16**, **17**, and **22** were evaluated for their cytotoxicity against several cancer cell lines. Compounds **10**, **11**, **12**, **13**, and **15** were tested for their antioxidant activity.

Compound **1** was isolated as yellow needles, positive to Dragendorff's test. Its molecular formula was determined as $C_{19}H_{17}O_6N$ on the basis of its HR-EI-MS spectrum (*m*/*z* 355.1058 $[M]^+$, Calcd 355.1055). The UV spectrum showed absorption at λ_{max} 230, 252, and 282 nm. The IR spectrum showed absorption bands at 1664, 1734, and 3421 indicating carbonyl and hydroxyl groups, respectively. The ^{1}H-NMR spectra revealed signals for two methoxy groups (δ_H 4.08, 4.14), two aromatic protons (δ_H 6.97, 8.25), and an *N*-methyl group (δ_H 3.75), which appeared at low field, reflecting an unusual dehydroaporphine moiety.[5,25)] The proton signals at δ_H 2.18, 2.73, 3.15, and 3.29 were ascribed to methylene protons at the 8,9-positions of the D-ring dehydroaporphine moiety (Fig. 3).[5)] The ^{13}C-NMR spectrum exhibited the presence of three methyl, two methylene, three methine, and eleven quaternary carbons. In comparison with the literature data,[25)] the di-ketone groups in 4,5-dioxoaporphines usually resonate at δ_C 178 and 157 ppm, respectively. Compound **1** showed the signals at δ_C 175.0 and 152.4 ppm, which are coincident with the assignments of 4,5-di-ketone groups. H-3 appeared at δ_H 8.25 indicating the existence of a carbonyl group at the peri-position. The HMBC spectra gave further support for the structure determination of **1**, the correlations between 1-OCH_3 and C-1, and 2-OCH_3 and C-2 confirmed the methoxy groups at C-1 and C-2. The correlations between H-3 and C-2/C-11c/C-4, and between N-CH_3 and C-5/C-6a indicated the ketone groups at C-4 and C-5. The significant NOESY correlation between H-7 and H-8 together with the aforementioned assignments also proved the carbonyl group was located at C-11. The above evidence and comparison with the spectral data reported for artacinatine (**18**), cepharadione-A, and aristolodione indicated the structure of **1** was 4,5-dioxoartacinatine.[25)] In our previous study, artacinatine (**18**) isolated from this plant with the same D-ring moiety had been evidenced by X-ray crystalline analysis. Compound **18** possesses a 10α-hydroxyl function. Com-



(1) (2)

Fig. 1. Structure of 4,5-Dioxoartacinatine (**1**) and 24-Methylenelanosta-7,9(11)-diene-3-one (**2**)



1 2

Fig. 2. Key COSY and Key HMBC Correlations for **1** and **2**

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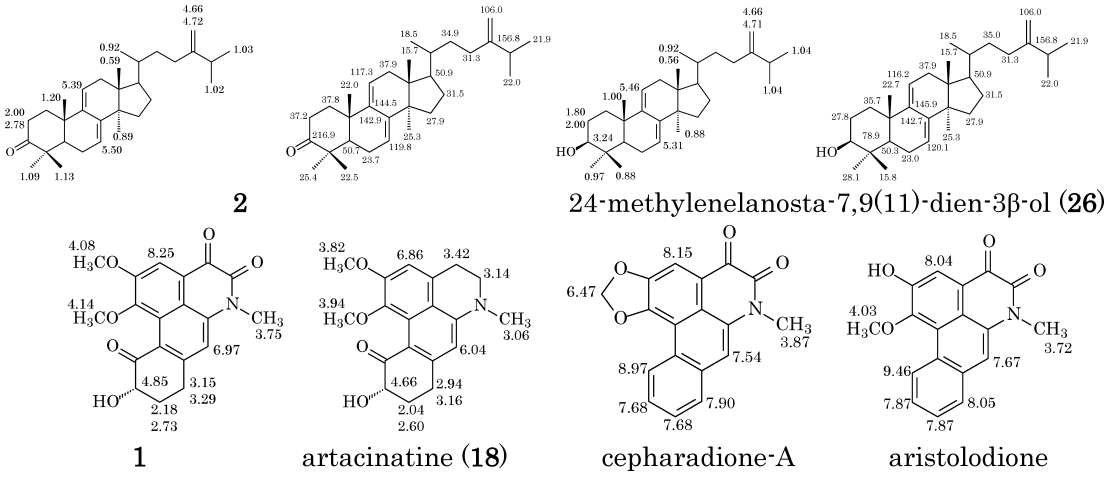

Fig. 3. ^{1}H- and ^{13}C- NMR Spectra Data of **1**, **2**, Artacinatine (**18**), 24-Methylenelanosta-7,9(11)-dien-3β-ol (**26**), Cepharadione-A, and Aristolodione

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for **1** and **2** in $CDCl_3$[a)]

	1			**2**				
	H	C		H	C		H	C
1		150.0	1	2.00 (2H, m)	37.8	17	1.60 (1H, m)	50.9
2		152.4	2	2.00 (1H, m) 2.78 (1H, td, J=14.8, 5.9 Hz)	37.2	18	0.59 (3H, s)	15.7
1-OCH_3	4.14 (3H, s)	61.8	3		216.9	19	1.20 (3H, s)	22.0
2-OCH_3	4.08 (3H, s)	52.6	4		47.5	20		36.2
3	8.25 (1H, s)	115.9	5	1.50 (1H, m)	50.7	21	0.92 (3H, d, J=6.4 Hz)	18.5
3a		125.0	6	2.00 (2H, m)	23.7	22	2.00 (1H, m), 2.30 (1H, m)	34.9
4		175.0	7	5.50 (1H, d, J=6.4 Hz)	119.8	23	1.70 (1H, m), 2.30 (1H, m)	31.3
5		156.9	8		142.9	24		156.8
6a		137.0	9		144.5	25	2.00 (1H, m)	33.8
N-CH_3	3.75 (3H, s)	30.5	10		37.2	26	1.03 (3H, d, J=2.4 Hz)	21.9
7	6.97 (1H, s)	112.1	11	5.39 (1H, d, J=6.0 Hz)	117.3	27	1.02 (3H, d, J=2.0 Hz)	22.0
7a		149.0	12	2.00 (2H, m)	37.9	28	1.09 (3H, s)	25.4
8	3.15 (1H, ddd, J=17.0, 5.2, 4.0 Hz) 3.29 (1H, ddd, J=17.0, 11.2, 5.2 Hz)	28.8	13		43.7	29	1.13 (3H, s)	22.5
9	2.18 (1H, m), 2.73 (1H, m)	35.3	14		50.3	30	0.89 (3H, s)	25.3
10	4.85 (1H, dd, J=11.2, 6.0 Hz)	72.8	15	1.30 (1H, m), 2.00 (1H, m)	27.9	31	4.66 (1H, s), 4.72 (1H, s)	106.0
11		200.1	16	1.40 (1H, m), 1.60 (1H, m)	31.5			
11a		146.0						
11b		115.0						
11c		118.0						

a) Chemical shift values are given in ppm, and *J* values in parenthese are given in Hz. Assignments were confirmed by ^{1}H–^{1}H COSY, HMQC, and HMBC experiment.

pound **1** shows the exact pattern as **18** in NMR assignment, thus, we predicted that **1** also has a 10α-hydroxyl function.

Compound **2** was obtained as a colorless solid. The UV spectrum showed characteristic absorptions at λ_{max} 242, 235 nm. The IR spectrum of **2** contained absorption for the carbonyl group at 1707 cm^{-1}. The EI-MS spectrum showed a molecular ion peak at *m*/*z* 436 and HR-EI-MS spectrum gave *m*/*z* 436.3708 for the $[M]^+$ ion (Calcd 436.3705) corresponding to the molecular formula $C_{31}H_{48}O$. The ^{1}H-NMR spectrum of **2** indicated three secondary methyl groups (δ_H 0.92, 1.02, 1.03), five tertiary methyl groups (δ_H 0.59, 0.89, 1.09, 1.13, 1.20), two olefinic protons (δ_H 5.39, 5.50), and geminal protons for one terminal double bond (δ_H 4.66, 4.72). The ^{13}C-NMR spectrum of **2** showed signals due to a 7,9(11)-conjugated diene at δ_C 119.8, 142.9, 144.5, and 117.3, eight methyls at δ_C 25.4, 25.3, 22.5, 22.0, 22.0, 21.9, 18.5, and 15.7, and a ketone at δ_C 216.9. Further evidence from COSY, HMQC, and HMBC spectra also confirmed the planar structure of **2**. The HMBC cross peaks between H-2 and C-3, and CH_3-28 and C-3 indicated the carbonyl group was located at C-3, the COSY correlations from H-15 to H-23 and HMBC cross peaks between CH_3-21 and C-17/C-20/C-22, CH_2-31 and C-23/C-24/C-25, and CH_3-26 and C-24/C-25/C-27 revealed the side chain was situated at C-17. The stereochemistry of **2** was deduced by NOESY experiments, the NOE correlation between H-5 and H-28 indicated that H-5 was assigned to be in a α orientation. According to the aforementioned evidence, analyses of 1D and 2D NMR spectra and comparison with data of 24-methylenelanista-7,9(11)-dien-3β-ol (**26**) (Fig. 3),[23)] confirmed that the structure of **2** was 24-methylenelanosta-7,9(11)-diene-3-one.

According to the previous literature, caryolane-1,9β-diol

(**4**), liriodenine (**16**), atherospermidine (**17**), (+)-syringaresinol (**22**), and 24-methylenelanosta-7,9(11)-dien-3β-ol (**26**) had significant cytotoxicity, anti-HIV activity, and anti-inflammatory activity.[26,27]

In biological assay, atherospermidine (**17**) and (+)-syringaresinol (**22**) show significant inhibition against several cancer cell lines,[28] including Hep G2 (human hepatocellular carcinoma) cell line with IC_{50} values of 0.97 and 0.35 μg/ml, respectively.

Flavonoids were reported to possess antioxidant and free radical scavenging activities.[29] Compounds **10**, **11**, **12**, **13**, and **15** were tested for their antioxidant activity.[30] Among them, artabotryside A (**10**), luteolin (**13**), and (+)-catechin (**15**) were found to be powerful scavengers of DPPH free radicals with IC_{50} values of 14.09, 15.32, and 5.55 μg/ml, respectively. Artabotryside A (**10**) showed a significant effect (IC_{50} 10.19 μg/ml) on scavenging hydroxyl radical and luteolin (**13**) showed good SOD-like activity (IC_{50} 24.52 μg/ml).

Experimental

Melting points were measured on a Yanagimoto micro-melting point apparatus and were uncorrected. The UV spectra were obtained on a Jasco V-530 UV/VIS spectrophotometer. The IR spectra were recorded on a Mattson Genesis II spectrophotometer. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded with Varian NMR spectrometers. LR-EI-MS were collected on a Finnigan POLARISQ mass spectrometer. HR-EI-MS were collected on a Bruker DALTONICS Apex II mass spectrometer. TLC analysis was carried out on Si gel GF_{254} pre-coated plates with detection using 50% H_2SO_4 followed by heating on a hot plate.

Plant Material Fresh stems of *A. uncunatus* were collected in Kaohsiung, Taiwan in February, 2004. The voucher specimen is deposited in the Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan.

Extraction and Isolation Air-dried stems (4.5 kg) of *A. uncinatus* were extracted with methanol at room temperature, the concentrated methanolic extract was partitioned with CH_3OH (H_2O : CH_3OH=7 : 3) and *n*-hexane, the CH_3OH layer was partitioned with CH_3OH (H_2O : CH_3OH=1 : 1) and EtOAc. The *n*-hexane residue was subjected to Sephadex LH-20 (42×4 cm) column chromatography, eluting with *n*-hexane : EtOAc=1 : 1, and the collected fractions were combined on the basis of their TLC characteristics to give 4 fractions. Fraction 1 was separated by CC over silica gel and eluted with gradient mixtures of $CHCl_3$/EtOAC/CH_3OH to give 20 fractions. Fraction 1-2 was further chromatographed on silica gel column chromatography and high performance liquid chromatography (HPLC), and three compounds were obtained: 24-methylenelanosta-7,9(11)-diene-3-one (**2**) (4.0 mg), spathulenol (**6**) (8.5 mg) , and β-caryophyllene-8*R*,9*R*-oxide (**9**) (2.2 mg). Fractions 1-3—1-5 were purified by recrystallization from CH_3OH to afford 24-methylenelanosta-7,9(11)-dien-3β-ol (**26**) (60.0 mg) and a mixture of β-sitosterol and stigmasterol (**29**, **30**) (407.0 mg), and subfraction 1-3-11 was further chromatographed on high performance liquid chromatography (HPLC) to afford phytol (**25**) (28.0 mg). Fraction 1-8 was chromatographed on Sephadex LH-20 column chromatography and high performance liquid chromatography (HPLC) to afford a mixture of (24*R*)-stigmasta-5-en-3β,7α-diol and (22*E*,24*S*)-stigmasta-5,22-dien-3β,7α-diol (**27**, **28**) (2.2 mg). Fraction 1-11 was chromatographed on silica gel column chromatography to afford atherospermidine (**17**) (9.2 mg). Fr. 1-18 was purified by recrystallization from EtOAc to afford a mixture of β-sitosteryl-3-*O*-β-D-glucoside and stigmasteryl-3-*O*-β-D-glucoside (**31**, **32**) (450.0 mg).

The EtOAc layer (101 g) was subjected to silica gel column chromatography and eluted with gradient mixtures of $CHCl_3$/CH_3OH. The collected fractions were combined into 15 fractions on the basis of TLC monitoring. Fraction 2 was further chromatographed on silica gel column chromatography and high performance liquid chromatography (HPLC) to give **1** (0.5 mg), 1-methoxy-9-caryolanol (**5**) (2.6 mg), (−)-*ent*-4β-hydroxy-10β-methoxyaromadendrane (**7**) (8.2 mg), 4β-hydroxy-10α-methoxyaromadendrane (**8**) (3.0 mg), liriodenine (**16**) (3.0 mg), artacinatine (**18**) (26.0 mg), and (+)-syringaresinol (**22**) (8.0 mg). Fraction 4 was chromatographed on silica gel column chromatography and preparative TLC to afford cloven-2β,9α-diol (**3**) (2.6 mg), caryolane-1,9β-diol (**4**) (3.0 mg), and (2*R*,3*R*)-3-hydroxyl-2-methylbutyrolactone (**23**) (12.0 mg). Fraction 5 was chromatographed on Sephadex LH-20 column chromatography to give apigenin (**12**) (3.1 mg) and 5-hydroxy-7,4′-dimethoxyflavone (**14**) (1.0 mg). Fraction 6 was further chromatographed on silica gel column chromatography and high performance liquid chromatography (HPLC) to afford luteolin (**13**) (8.0 mg) and *N*-*P*-coumaroyltyramine (**21**) (7.0 mg). Fraction 7 was further chromatographed on silica gel column chromatography and high performance liquid chromatography (HPLC) to give (+)-catechin (**15**) (5.0 mg), (−)-asimilobine (**19**) (2.5 mg), and tetrahydrofuran-4-methylidene-3-ol (**24**) (32.0 mg). Fractions 10 and 12 were chromatographed on silica gel column chromatography to afford artabotryside A, (**10**) (45.0 mg), artabotryside B (**11**) (109.0 mg), and (−)-artavenustine (**20**) (45.0 mg).

4,5-Dioxoartacinatine (**1**): Yellow needles. mp 167—169 °C. $[\alpha]_D$ +52.2° (c=0.03, CH_3OH). ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz) see Table 1. IR ν_{max} cm^{-1}: 3421, 2924, 1734, 1664, 1461, 1142, 1055. UV λ_{max} nm: 396, 355, 345, 282, 252, 230. FAB-MS *m*/*z* (rel. int. %): 356 ($[M+H]^+$). EI-MS (70 eV) (rel. int. %): *m*/*z*=328 (33), 283 (28), 174 (34), 146 (36), 106 (79), 98 (79), 91 (100). HR-EI-MS: Calcd for $C_{19}H_{17}O_6N$ *m*/*z* $[M]^+$ 355.1055, found 355.1058.

24-Methylenelanosta-7,9(11)-diene-3-one (**2**): Colorless solid. mp 164—166 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz) see Table 1. IR ν_{max} cm^{-1}: 2930, 1707, 1378. UV λ_{max} nm: 242, 235. EI-MS (70 eV) (rel. int. %): *m*/*z*=436 (14), 421 (19), 309 (100), 268 (83), 171 (34). HR-EI-MS: Calcd for $C_{31}H_{48}O$ *m*/*z* $[M]^+$ 436.3705, found 436.3708.

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